

AD \_\_\_\_\_

Award Number: W81XWH-04-1-0143

TITLE: Lipoxygenase, Angiogenicity, and Prostate Cancer Radioresistance

PRINCIPAL INVESTIGATOR: Daotai Nie, Ph.D.

CONTRACTING ORGANIZATION: Southern Illinois University School of Medicine  
Springfield, IL 62702

REPORT DATE: January 2006

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. <b>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</b>					
1. REPORT DATE 01-01-2006		2. REPORT TYPE Annual		3. DATES COVERED 1 Jan 2005 – 31 Dec 2005	
4. TITLE AND SUBTITLE  Lipoxygenase, Angiogenicity, and Prostate Cancer Radioresistance				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-04-1-0143	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)  Daotai Nie, Ph.D.				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  Southern Illinois University School of Medicine Springfield, IL 62702				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES Original contains colored plates: ALL DTIC reproductions will be in black and white.					
14. ABSTRACT Radiotherapy is a prevalent modality for the treatment of prostate tumor. Although radiation is capable of eradicating localized prostate tumors, nearly 30% of patients treated with potentially curative doses relapse at the sites of irradiation. Therefore, there is an imperative need to improve the success rate of radiotherapy for PCa. This proposal is focused on a role of 12-lipoxygenase (LOX) in modulating the radiation response of PCa cells. 12-LOX catalyzes the formation of 12(S)-hydroxyeicosatetraenoic acid (HETE). Our studies suggest an involvement of 12-LOX in radioresistance of PCa cells. It is our hypothesis that an increase in 12-LOX expression/activity may lead to an increased resistance in tumors to radiation treatment. We also hypothesize that VEGF is an important intermediary for 12-LOX mediated radioresistance in PCa. We intend to define the role of 12-LOX in radioresponse in PCa. 12-LOX will be overexpressed in LNCaP and DU145 cells. Then we will study whether an increase in 12-LOX expression in LNCaP and DU145 cells can enhance their resistance to radiotherapy. We also propose to study whether VEGF is required by 12-LOX to enhance PCa radioresistance through blockade of VEGF activity with a neutralizing antibody. Finally, we will evaluate whether BHPP, a 12-LOX inhibitor, can be used to sensitize prostate tumors to radiotherapy.					
15. SUBJECT TERMS Prostate cancer, radiotherapy, 12-lipoxygenase, apoptosis, angiogenesis					
16. SECURITY CLASSIFICATION OF:			UU	18. NUMBER OF PAGES  15	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

## Table of Contents

<b>COVER.....</b>	<b>1</b>
<b>Introduction.....</b>	<b>4</b>
<b>BODY.....</b>	<b>4</b>
<b>Conclusions.....</b>	<b>12</b>
<b>Reportable Outcomes.....</b>	<b>12</b>
<b>Appendix .....</b>	<b>13</b>

## INTRODUCTION

Prostate cancer (PCa) is one of most common cancers affecting American men. Radiotherapy is a prevalent modality for the treatment of prostate tumor. Although radiation is capable of eradicating localized prostate tumors, nearly 30% of patients treated with potentially curative doses relapse at the sites of irradiation. Therefore, there is an imperative need to improve the success rate of radiotherapy for PCa.

This proposal is focused on a role of 12-lipoxygenase (LOX) in modulating the radiation response of PCa cells. 12-LOX catalyzes the formation of 12(S)-hydroxyeicosatetraenoic acid (HETE) and it has been implicated in PCa growth and progression. Our studies suggest an involvement of 12-LOX in radioresistance of PCa cells. It is our hypothesis that an increase in 12-LOX expression/activity may lead to an increased resistance in tumors to radiation treatment. Conversely, a downregulation of 12-LOX expression or activity can sensitize PCa cells to radiotherapy. We also hypothesize that VEGF is an important intermediary for 12-LOX mediated radioresistance in PCa. Here we propose to expand our study on the role for 12-LOX in radioresponse in PCa. 12-LOX will be overexpressed in LNCaP and DU145 cells. Then we will study whether an increase in 12-LOX expression in LNCaP and DU145 cells can enhance their resistance to radiotherapy. We also propose to study whether VEGF is required by 12-LOX to enhance PCa radioresistance through blockade of VEGF activity with a neutralizing antibody. Finally, we will evaluate whether BHPP, a 12-LOX inhibitor, can be used to sensitize prostate tumors to radiotherapy. The following specific aims are proposed:

Aim 1. Expand the study on the role of 12-LOX in radioresponse in PCa cells.

Aim 2. Determine whether or not stimulation of VEGF is required by 12-LOX to enhance radioresistance in vitro and in vivo.

Aim 3. Evaluate whether or not 12-LOX inhibitor BHPP can sensitize prostate tumors to radiation in vivo.

## BODY OF REPORT

### KEY RESEARCH ACCOMPLISHMENT

- 1 provisional patent application filed
- 1 review article published
- 1 research article in submission
- 1 review article in press
- 2 abstracts published

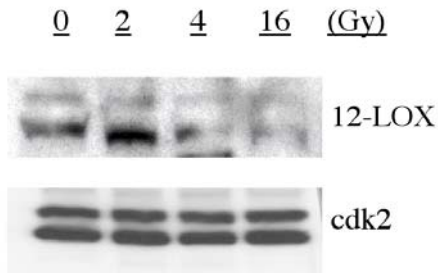
### PROGRESS

**Task 1.** Expand the study of the role for 12-LOX in radioresponse in prostate cancer cells. Months 1 - 18:

In this aim, the regulation of 12-LOX levels by IR will be studied in a number of prostate cancer cell lines. The radiosensitizing effects of 12-LOX inhibitors in more PCa cell lines and whether 12(S)-HETE can protect them from radiation will be studied. This task has been largely completed, with findings summarized below.

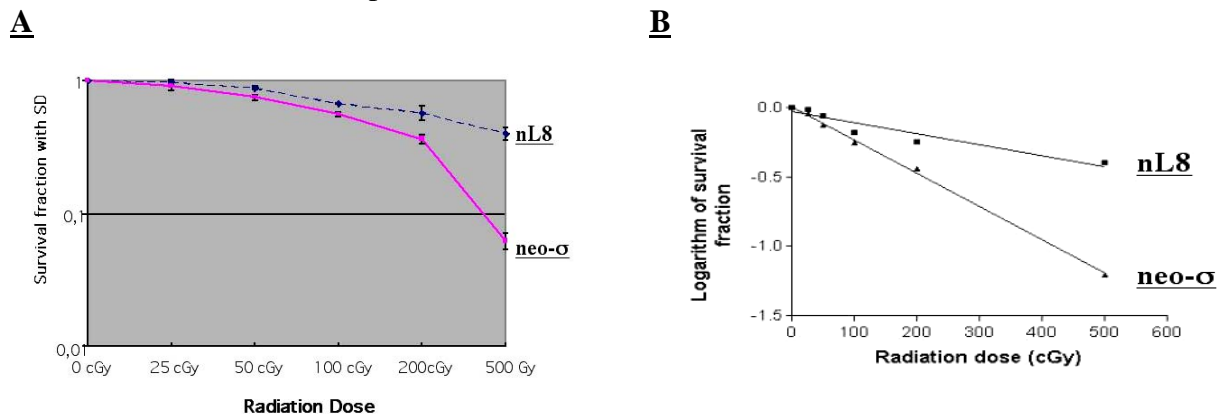
To study whether or not radiation regulates 12-LOX, we subjected LNCaP cells to radiation of different doses and cultured in serum containing media (RPMI1640-10%FBS) for 16 h. LNCaP cells were selected because they express 12-LOX consistently in culture (Nie et al., 2001). As shown in **figure 1**, low-

dose radiation (200 cGy) increased the protein level of 12-LOX, suggesting that the gene expression of 12-LOX was stimulated by low dose radiation. Interestingly, at higher doses (400 and 1600 cGy), the steady state levels of 12-LOX were reduced. The reduction of 12-LOX level is not due to cell death because we did not notice any significant cell death 16 h after irradiation at doses indicated. The drastic changes in 12-LOX levels as a function of radiation imply that 12-LOX is probably involved in radiation response.



**Figure 1.** Effect of radiation on 12-LOX expression in prostate carcinoma LNCaP cells. Note the stimulation of 12-LOX expression by low dose radiation (200 cGy) but at higher doses, 12-LOX expression was reduced (400 cGy and 1600 cGy). The level of cdk2 is included for reference for sample loading.

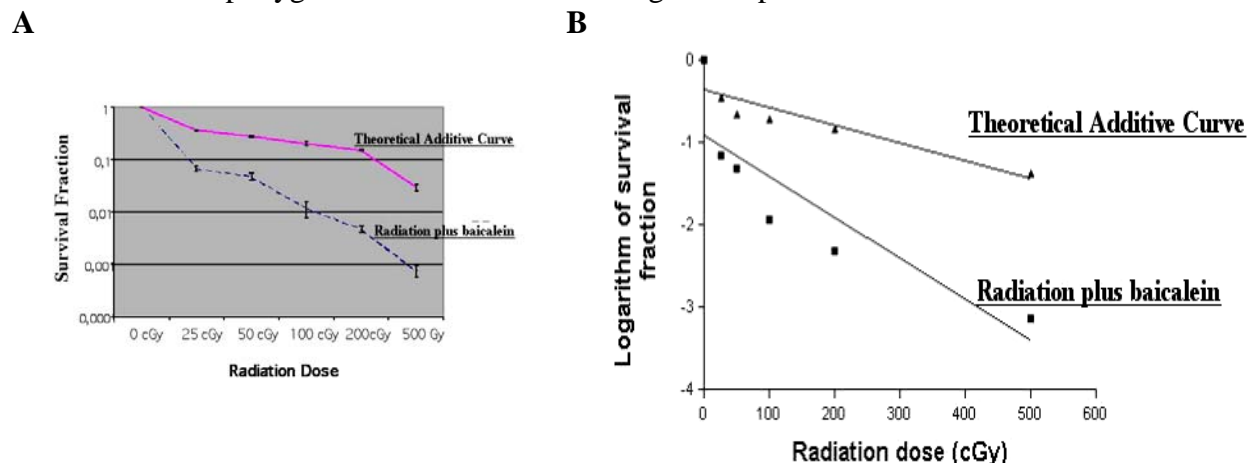
To determine whether 12-LOX plays a role in radioresponse of carcinoma cells, we used a panel of PC-3 cell sublines that were stably transfected with an expression construct of platelet-type 12-LOX. The isolated clones had an increased 12-LOX expression and 12(S)-HETE biosynthesis (Nie et al., 1998). Next, we examined the effects of increased expression of 12-LOX on colony formation of carcinoma cells after radiation. As shown in **Figure 2**, nL8, a 12-LOX overexpressing clone (Nie et al., 1998), presented strong radioresistance when compared to its vector control, neo- (**Figure 2 A**), as indicated by enhanced clonogenic survival. Regression analysis indicated a significant difference in radioresistance between nL8 and neo- ( $P < 0.01$ ) (**Figure 2 B**). The data suggest that increased expression or activity of 12-LOX enhances radioresistance in prostate carcinoma cells.



**Figure 2.** 12-Lipoxygenase enhances radioresistance of PC-3 cells as indicated by colony formation assay. **A**, Increased clonogenic survival by enhanced expression of 12-LOX in PC-3 cells. nL8, a 12-LOX overexpressing clone of PC-3 cells; neo-σ, vector control. **B**, Regression analysis.  $P < 0.01$ .

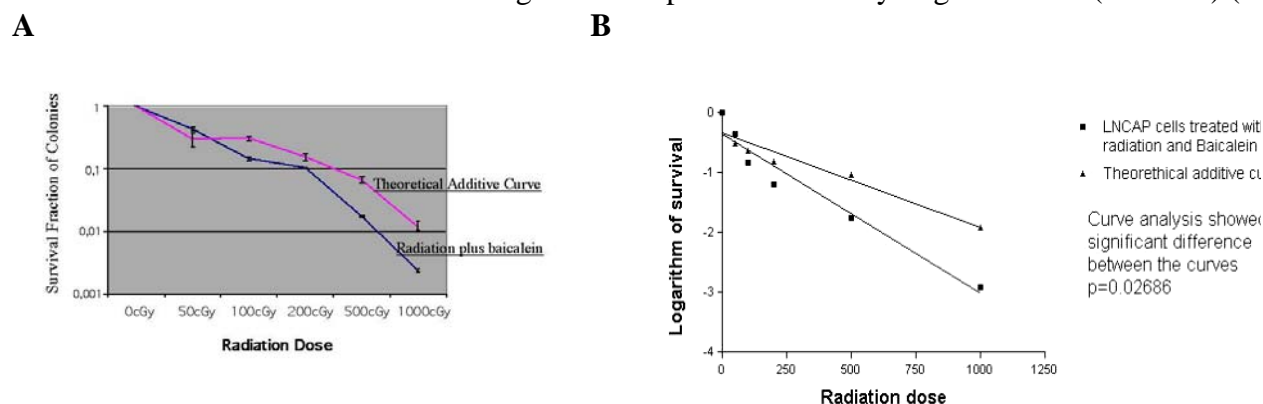
Next we studied whether baicalein also sensitizes androgen-independent PCa cells to radiation therapy as it did in LNCaP cells. PC-3 cells were treated with 7.5  $\mu$ M baicalein for two hours before initiation of radiation. As shown in **figure 3 A and B**, baicalein and radiation, when combined, have super

additive or synergistic inhibition on the colony formation of PC3 cells ( $P < 0.01$ ). The data suggest that inhibitor of 12-lipoxygenase also sensitizes androgen independent PC-3 cells to radiation.



**Figure 3.** Radiosensitization of androgen independent PC-3 cells by baicalein. **A.** 12-LOX inhibitor baicalein sensitizes PC3 cells to radiation as indicated by colony formation assay. **B.** Regression analysis.  $P = 0.0086$ .

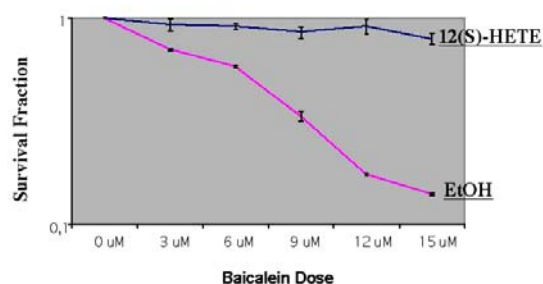
Next we examined whether inhibition of 12-LOX can modulate the radioresponse of PCa cells. First we examined the effect of baicalein, a select inhibitor of 12-LOX, on radioresponse of androgen dependent LNCaP cells. We treated LNCaP cells with 7.5  $\mu\text{M}$  baicalein for 2 hrs before initiation of radiation. As shown in **figure 4 A**, baicalein and IR, when combined, have super additive or synergistic inhibitory effect on the colony formation of LNCaP cells. Regression analysis indicates that combined treatment of LNCaP cells with radiation and baicalein has significant super-additive or synergistic effect ( $P < 0.05$ ) (**Figure 4 B**).



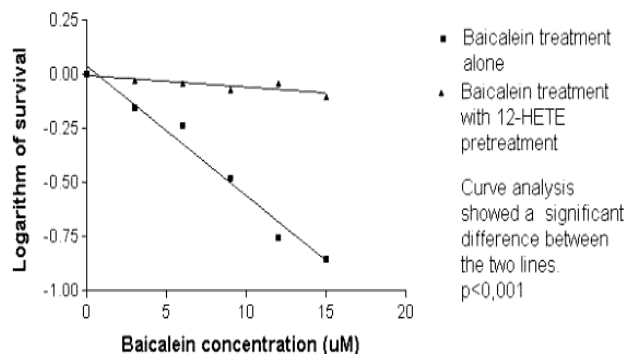
**Figure 4.** Radiosensitization of LNCaP cells by a 12-LOX inhibitor, baicalein. **A.** 12-LOX inhibitor baicalein sensitizes LNCaP cells to IR as indicated by colony formation assay. Refer to the General Method in section D for detailed description of the calculation of theoretical additive curve and other statistical calculation. **B.** Regression analysis.  $P = 0.02688$ .

The main stable arachidonate product of 12-LOX is 12(S)-HETE. To study whether or not 12(S)-HETE modulates radioresistance of carcinoma cells, we treated PC-3 cells with graded levels of baicalein (0, 3, 6, 9, 12, and 15  $\mu\text{M}$ ), in the presence or absence of 300 nM of 12(S)-HETE, for 2 h before irradiation (200 cGy). As shown in **Figure 5 A**, baicalein sensitized PC-3 cells to radiation in a dose dependent manner. The radiosensitization of PC-3 cells by baicalein was completely abolished by exogenously added 12(S)-HETE (**Figure 5 A and B**,  $P < 0.01$ ). Therefore, radiosensitization of PC-3 cells by baicalein is dependent on the absence of 12(S)-HETE. The results further suggest the involvement of the 12-LOX activity in radioresistance of prostate carcinoma cells.

A

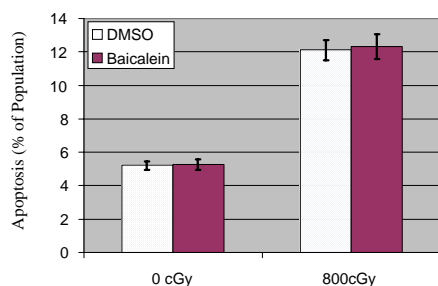


B

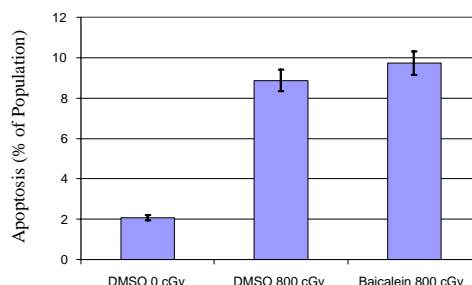


**Figure 5.** Radiosensitization of PC-3 cells by baicalein was abolished by exogenously added 12(S)-HETE. **A.** Attenuation of baicalein radiosensitization of PC-3 cells by 12(S)-HETE as indicated by colony formation assay. The radiation dose used was 200 cGy. **B.** Regression analysis.  $P < 0.001$ .

To study whether 12-LOX inhibitors can also sensitize normal prostate epithelial cells to radiation, we treated human normal prostate epithelial cells (purchased from Clonetics, San Diego, CA) with 7.5 μM baicalein 2 h before radiation (800 cGy). The cells are harvested 36 h after radiation for evaluation of apoptosis using a commercial flow cytometric assay kit based on TUNEL staining (APO-DIRECT, Pharmingen, San Diego, CA). We use apoptosis, rather than clonogenic survival, as the end point for potential radiosensitization of normal prostate epithelial cells by 12-LOX inhibitors. The rationale is that unlike prostate cancer cells, normal prostate cells have limited ability to proliferate and form colonies. As shown in **Figure 6** and **Figure 7**, the presence of baicalein did not potentiate radiation-elicited apoptosis either in normal prostate epithelial cells or in human normal skin fibroblast. The lack of radiosensitization by 12-LOX inhibitor in normal prostate epithelial cells may be due to the low or absence of 12-LOX expression (Gao et al., 1995).

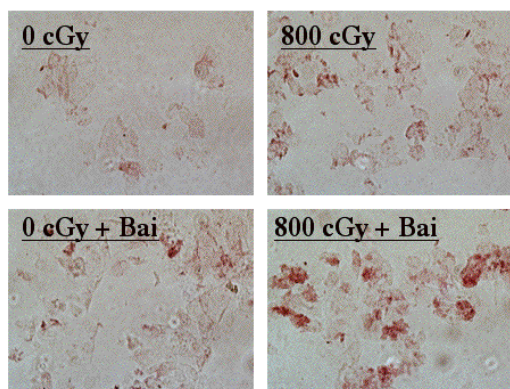


**Figure 6.** Lack of radiosensitization of baicalein, a 12-LOX inhibitor, in normal prostate epithelial cells. Note the increase in apoptosis after radiation (800 cGy) and the absence of effect of baicalein treatment on apoptosis, regardless of radiation.



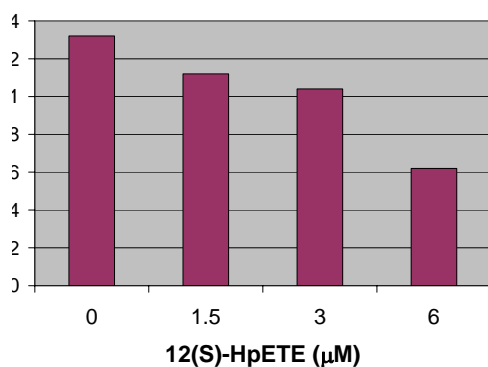
**Figure 7.** Lack of radiosensitization of baicalein in normal human skin fibroblast.

Since 12-LOX inhibitors can induce apoptosis, radiosensitization of tumor cells by baicalein is likely mediated by potentiation of apoptosis. To study this possibility, we evaluated the level of cleaved caspase-3, the activated form of caspase-3. As shown in **Figure 8**, combined treatment of A431 cells had highest level of caspase-3 activation.



**Figure 8.** Levels of cleaved caspase-3 16 h after baicalein and radiation treatment. Cells were fixed and immunostained for cleaved (activated) caspase-3 using standard ABC procedure. Blown staining (dark spots if black and white print) indicates positive staining.

Critical for apoptotic processes, caspases are cysteine-dependent and sensitive to oxidation, hence, high levels of lipid peroxide from 12-LOX may lead to their inactivation. To study this possibility, we examined whether 12(S)-HpETE can inhibit the activity of Caspase-3, an effector caspase, which can cleave a broad spectrum of cellular targets. As shown in **Figure 9**, 12(S)-HpETE inhibited caspase-3 activity in a dose-dependent manner.



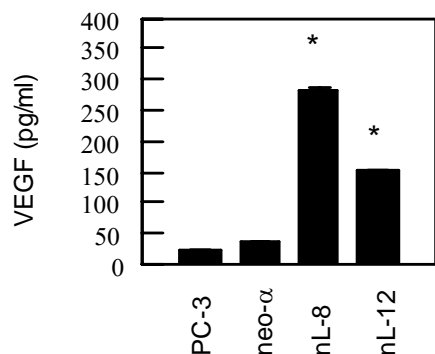
**Figure 9.** Inhibition of Caspase-3 activity by 12(S)-HpETE. Active caspase-3 (purchased from Biomol) were incubated with graded levels of 12(S)-HpETE for 10 min, before addition of substrate DEVD-pNA. After further 30 min of incubation, the cleavage of DEVD-pNA was measured at 405 nm and expressed as absolute unit. The results represent two independent experiments.

**Task 2. Determine whether or not stimulation of VEGF is required by 12-LOX to enhance radioresistance in vitro and in vivo.**

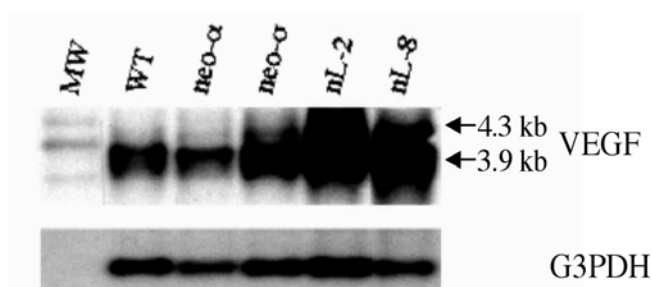
We will use a VEGF neutralizing antibody to study whether VEGF is required for 12-LOX mediated radioresistance in PC-3 cells. Matrigel implantation model will be used to assess 12-LOX mediated radioresistance in vivo and to study the role of VEGF in this process. This task has been initiated, with the following preliminary findings:

To study whether 12-LOX can regulate VEGF expression, we measured VEGF levels in culture supernatants from 12-LOX transfected PC-3 cells (nL-8 and nL-12) and their vector controls. As shown in **Figure 10**, increased expression of 12-LOX enhanced VEGF expression. Northern blot analysis revealed an increase in the levels of VEGF mRNA in 12-LOX transfected PC-3 cells (nL-2 and nL-8) (**Figure 11**).



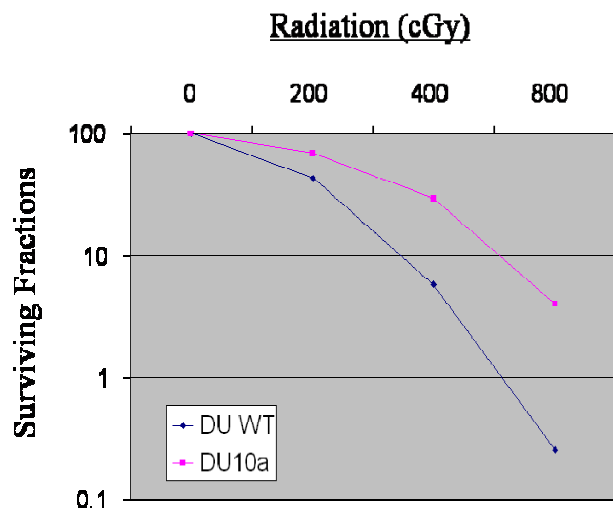


**Figure 10.** Increased VEGF Expression in 12-LOX Transfected PC-3 Cells. \*,  $P < 0.01$ .



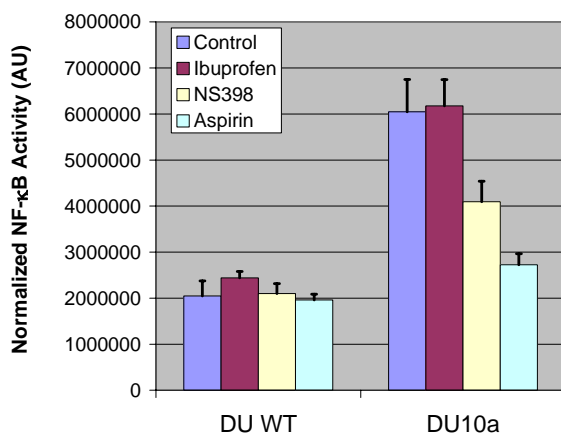
**Figure 11.** Northern Blot Analysis of VEGF mRNA Levels. Poly(A)+RNA were isolated and the 12-LOX mRNA levels were analyzed with labeled VEGF cDNA. The membrane was then stripped and probed for G3PDH as a loading control.

As part of our effort to study how prostate cancer cells survive clonogenically from radiation treatment, we subjected PCa cells to radiation treatment (800 cGy) and the clonogenically survived cells were isolated and propagated. As shown in **Figure 12**, a subline of DU145 cells (DU10a), presented a much higher resistance to subsequent radiation treatment. Similar results were also obtained in PC-3 cells. The data suggest that prostate cancer cells, after surviving potential lethal dose of radiotherapy, become more resistant to subsequent radiation treatment.



**Figure 12.** Increased radioresistance of DU145 cells after surviving radiotherapy. DU10a are DU145 cells that have survived a potential lethal dose of radiation (800 cGy). DUWT are parental DU145 cells that have survived from sham radiation.

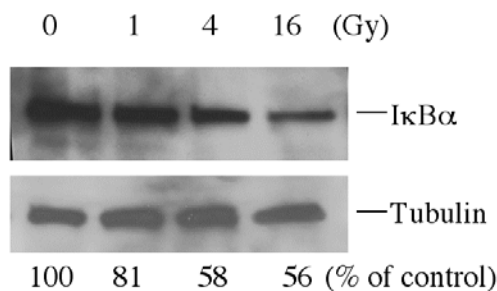
Next we examined whether there is a change in NF- $\kappa$ B activity, which is known for its role in angiogenesis and tumor growth, in tumor cells surviving radiation treatment. As shown in **Figure 13**, there was a four fold of increase in NF- $\kappa$ B promoter activity in DU10a, as compared to the control, DU145 cells. The data suggest that there is a *sustained* elevation in NF- $\kappa$ B activity in PCa cells that clonogenically survived from radiation treatment.



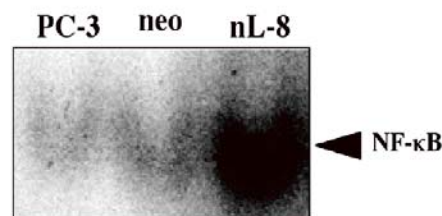
**Figure 13.** Increased NF- $\kappa$ B activity in clonogenically survived DU145 cells (DU10a) and its downregulation by aspirin and NS398. Cells were transfected with a reporter gene construct under NF- $\kappa$ B promoter and treated with ethanol (control), ibuprofen (1 mM), NS398 (20  $\mu$ M), or aspirin (0.5 mM) for 18 h before luciferase assay. Note the downregulation of NF- $\kappa$ B activity in DU10a cells by NS398 and aspirin.

Interestingly, the sustained activation of NF- $\kappa$ B in radioresistant DU10a cells was markedly reduced by aspirin treatment (**Figure 13**). The ability of this widely used OTC drug to downregulate the sustained activation of NF- $\kappa$ B in clonogenically survived PCa cells raises an exciting possibility, of using inhibitors of NF- $\kappa$ B, such as sodium salicylates (aspirin), to overcome PCa radioresistance. Studies are ongoing to study whether aspirin can reduce the expression of VEGF and also enhances the efficacy of radiotherapy.

Radiation has been demonstrated to activate NF- $\kappa$ B as part of the immediate early response. We also found that IR can increase NF- $\kappa$ B promoter activities in prostate carcinoma PC-3 and DU145 cells in a dose dependent manner (data not shown) and this activation was associated by a reduction in the level of I $\kappa$ B $\alpha$  (**Figure 14**).



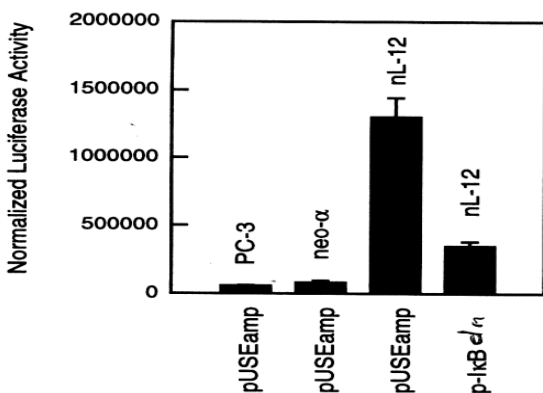
**Figure 14.** Reduction of I $\kappa$ B $\alpha$  levels in prostate carcinoma DU145 cells after IR. Shown here is the level of I $\kappa$ B $\alpha$  level 16 h after IR as revealed by western blot. The level of tubulin was included as a loading control.



**Figure 15.** Effect of 12-lipoxygenase overexpression on NF- $\kappa$ B activity. EMSA was performed on nuclear extracts of untransfected PC-3, neo, and 12-LOX transfected cells (nL8). Note the increase in NF- $\kappa$ B DNA binding activity in nL8.

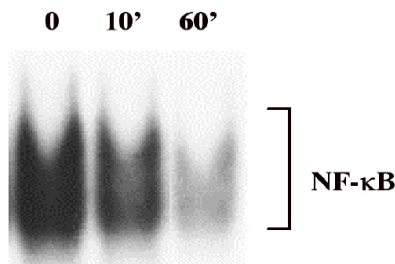
Radiation also rapidly increases the enzymatic activity of 12-LOX. We examined whether or not 12-lipoxygenase regulates NF- $\kappa$ B activity in prostate cancer cells using electomobility shift assays (EMSAs), western blotting for I $\kappa$ B $\alpha$ , and transcriptional activity with luciferase reporter assay. Nuclear protein extracts of 12-LOX transfected cells (nL-8) showed significant constitutive activation of NF- $\kappa$ B compared to the vector control cells (neo- $\alpha$ ) or the untransfected PC-3 cells (**Figure 15**).

This activation was further confirmed by the increased transcriptional activity of the luciferase reporter construct, driven by NF- $\kappa$ B, in nL-12 cells (**Figure 16**). This increase in transcriptional activity observed in 12-LOX transfected cells was nearly abolished upon co-transfection of a mutant of I $\kappa$ B $\alpha$  that is resistant to proteolytic degradation purchased from Upstate Biotechnonogy (Lake Placid, NJ) (**Figure 16**). Activation of NF- $\kappa$ B involves phosphorylation and eventual degradation of I $\kappa$ B protein before NF- $\kappa$ B could bind to DNA. Western blot analysis of whole cell protein extracts from neo- $\alpha$ , nL-8, and nL-12 cells showed a dramatic decrease in I $\kappa$ B $\alpha$  in nL-8 and nL-12 cells (**Figure 17**, in previous page). These results strongly suggest that overexpression of 12-LOX induces NF- $\kappa$ B activity by a mechanism involving proteolytic degradation of I $\kappa$ B $\alpha$ .

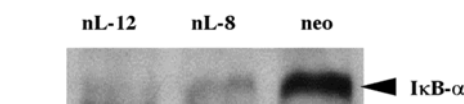


**Figure 16.** Effect of 12-lipoxygenase overexpression on NF- $\kappa$ B activity. The cells (PC-3, neo, and a clone of 12-LOX transfected PC-3, nL-12) were transfected with NF- $\kappa$ B-luciferase and LacZ reporters (pUSEamp) and the reporter activities measured. To elucidate the role of I $\kappa$ B $\alpha$  in 12-LOX activation of NF- $\kappa$ B, cells were also transfected with a dominant negative mutant I $\kappa$ B $\alpha$  construct (p-I $\kappa$ Bdn), or its control vector pUSEamp. The normalized data shown is an average of three experiments with standard deviation. LacZ expression was used for normalization.

Next we used BHPP, a select inhibitor of 12-LOX (Nie et al., 2000), to study the role of the enzymatic activity of 12-LOX in NF- $\kappa$ B activation. DNA binding activity of NF- $\kappa$ B was greatly decreased upon exposure to 20  $\mu$ M BHPP for 60 min (**Figure 18**). The results show the participation of the 12-LOX enzymatic activity in NF- $\kappa$ B activation.

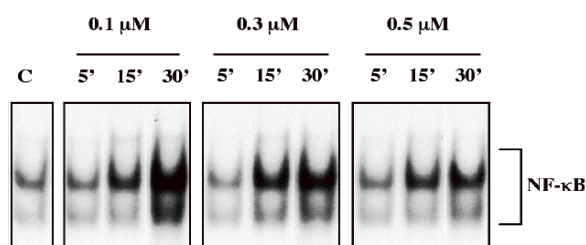


**Figure 18.** Inhibition of NF- $\kappa$ B activation in 12-Lipoxygenase transfected PC-3 cells (nL8) by BHPP, a select 12-LOX inhibitor

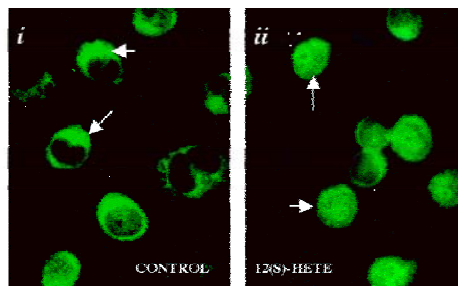


**Figure 17.** Reduction of I $\kappa$ B $\alpha$  levels by overexpression of 12-LOX in PC-3 cells. The levels of I $\kappa$ B $\alpha$  were evaluated by western blot analysis in cell lysates from 12-LOX overexpressing PC-3 cells (nL-8 and nL-12) and their vector control (neo).

To further study the involvement of 12(S)-HETE in the activation of NF- $\kappa$ B, we evaluated the DNA binding activity of NF- $\kappa$ B when PC-3 cells were treated with 12(S)-HETE. As shown in **figure 19**, 12(S)-HETE can modulate NF- $\kappa$ B DNA binding activity in a dose- and time-dependent manner. Further, the increased NF- $\kappa$ B DNA binding activity was accompanied by the nuclear translocation of NF- $\kappa$ B from cytosol to the nucleus, as revealed by immunocytochemical analysis of the p65 subunit of NF- $\kappa$ B (**Figure 20**).



**Figure 19.** Effect of 12(S)-HETE on the activation of NF- $\kappa$ B in PC-3 cells. Time and dose dependent activation of NF- $\kappa$ B DNA binding activity by 12(S)-HETE. EMSA was performed on the nuclear extracts of the treated PC-3 cells. The cells were incubated with serum-free RPMI medium containing the amounts of 12(S)-HETE shown for the indicated time and subjected to EMSA.



**Figure 20:** Immunofluorescent localization of NF- $\kappa$ B in PC-3 cells with and without 12(S)-HETE treatment. Cells were treated with 100 nM 12(S)-HETE or buffer for 10 min and immunostained. Staining was predominantly present in the cytoplasm of untreated control cells (Left panel, arrows) and nuclear staining increased considerably in 12(S)-HETE treated cells (Right panel, arrows).

**Task 3. Evaluate whether or not 12-LOX inhibitor BHPP can sensitize prostate tumors to radiation in vivo.** We will evaluate whether BHPP, a 12-LOX inhibitor, can be used to sensitize xenografted prostate tumors to radiotherapy.

The task is just started and in the process of being implemented.

## SUMMARY/CONCLUSIONS:

Our studies found that 12-LOX promotes the resistance of prostate cancer cells toward radiation treatment. We also found when combined, 12-LOX inhibitors and radiation had synergistic effects in killing PCa cells and this was accompanied by an increase in the level of the active form of caspase-3. Our studies suggest that 12-LOX inhibitors are promising radiosensitizer and further work need to be done to determine the mechanism of radiosensitization and the efficacy of 12-LOX inhibitors in sensitizing prostate tumors to radiation treatment.

## REPORTABLE OUTCOMES

- Review article published.  
Nie D, Honn KV. Eicosanoid regulation of angiogenesis in tumors. *Semin Thromb Hemost.* 2004 30:119-25.
- Review article in Press.  
Nie D. Cyclooxygenases and Lipoxygenases in Cancer. *Frontier in Biological Science* 2006.
- Research article in revision

Nie, D., Y Chen, Y Qiao, A Zacharek, K Tang, J Milanini, G Pages, D Grignon, and KV Honn. Arachidonate 12-Lipoxygenase regulates the expression of vascular endothelial growth factor in a PI3 kinase dependent pathway.

- Abstract published.  
Krishnamoorthy, S., K. R. Maddipati, D. Nie, and K. V. Honn. 12-Lipoxygenase in hypoxia and hypoxia-induced angiogenesis. Proc. Amer. Assoc. Cancer Res. 45: #3591, 2004.
- Abstract published.  
Nie, D., Y. Qiao, A. Zacharek, and K. V. Honn. Blockade of NF-kB sensitizes prostate cancer cells to ionizing radiation. Proc. Amer. Assoc. Cancer Res. 45: #1290, 2004.
- Patent applied. A provisional patent application, entitled “12-Lipoxygenase inhibitors as radiosensitizer for prostate cancer” has been filed.
- Development of animal models: No.

## **Appendix**

### **1. Provisional Patent Application**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**PROVISIONAL APPLICATION FOR UNITED STATES LETTERS PATENT**

Express Mail Label No.	:	ER985571632US
Date Deposited	:	June 16, 2005
Attorney Docket No.	:	RM-WST
No. of Dwg. Figs./Sheets	:	16/12
No. of Claims -		
Independent	:	n/a
Total	:	n/a

**Inhibitors of 12-Lipoxygenase as Radiosensitizers  
for Radiation Treatment of Cancer**

by

Daotai Nie

and

Kenneth Honn

**ROHM & MONSANTO, P.L.C.**

*Attorneys at Law*

660 WOODWARD AVENUE, SUITE 1525

DETROIT, MICHIGAN 48226

TELEPHONE (313) 965-1976

TELECOPIER (313) 965-1951



**ROHM & MONSANTO, P.L.C.**

INTELLECTUAL PROPERTY LAWYERS

**First National Building**

**660 Woodward Avenue, Suite 1525**

**DETROIT, MICHIGAN 48226**

**BENITA J. ROHM**  
REGISTERED PATENT ATTORNEY  
UNITED STATES PATENT AND TRADEMARK OFFICE

ADMITTED TO PRACTICE  
STATE OF NEW JERSEY  
STATE OF MICHIGAN

TELEPHONE (313) 886-1966

TELECOPIER (313) 886-3130

OF COUNSEL:  
MILLER, CANFIELD, PADDOCK & STONE, P.C.

November 2, 2005

*Please respond to:*  
P.O. Box 15402  
Detroit, MI 48215-0402

Ms. Laura Johnston  
Assistant General Counsel  
**Wayne State University**  
4249 Faculty/Administration Building  
656 West Kirby  
Detroit, MI 48202

**Re:** U.S. Provisional Patent Appln S.N. 60/691,452 filed June 16, 2005  
entitled *Inhibitors of 12-Lipoxygenase as Radiosensitizers for Radiation  
Treatment of Cancer*  
by Daotai Nie and Kenneth Honn  
Our Ref.: RM. WST                      WSU File No. 04-678

Dear Ms. Johnston:

We enclose for your files a copy of a provisional patent application that we filed as a precautionary measure on **June 16, 2005** in order to prevent an accidental publication of the subject matter. This application was assigned serial number **60/691,452**.

As understood, however, the subject matter still has not be published. It is our plan to prepare and file a second provisional patent application, if warranted, or to convert this application to a non-provisional, if authorized. The second application should incorporate any new data from in vivo animal testing as well as any new developments. We specifically defined suitable 12-LOX inhibitors to baicalein, and of course the 300 hydroxamic acid derivatives in the Biomide library, citing Biomide patent nos. 5,234,933 and 5,292,884. It may be helpful, however, to provide the identities of the compounds used in the *in vitro* and *in vivo* studies, as well as to point out specifically preferred compounds.

We recommend keeping the serial number and the filing date confidential. In addition to the foregoing, the application itself should be kept in confidence. However, the University is now entitled to mark any product or equipment incorporating the subject matter of the patent application, or any instruction manuals, or the like, describing the method of the present invention, with "pat. pending" or "patent pending."

We forwarded an Assignment from the inventors to Wayne State to the United States Patent and Trademark Office for recordation when the application was filed. The Patent Office has not yet